SUPPLEMENTAL INFORMATION

Construction and Use of New Cloning Vectors for the Rapid Isolation of Recombinant Proteins from *Escherichia coli.*

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Running title: New plasmids for protein overproduction and isolation

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MATERIALS AND METHODS

Plasmid and strain construction. Restriction endonucleases *Nde*I, *Xba*I *Bpu*1102I, *Nhe*I, *Nco*I, *Kpn*I, Klenow fragment, and T4 DNA Ligase were purchased from Fermentas. Restriction endonucleases *Eco*RI, *Spe*I, *Hind*III, *Not*I, and *Sac*I were purchased from Promega and were used as per manufacturer's instructions. All restriction digests were resolved by agarose gel electrophoresis and gel extracted with the QIAquick Gel Extraction Kit (Qiagen). DNA fragments were introduced into plasmids by annealing the respective single-stranded DNA oligonucleotides and ligation into the respective plasmid at the restriction sites indicated. Invitrogen's One Shot® TOP10 Chemically Competent *E. coli* cells were used for all cloning work unless except in the construction of plasmids pTEV3 and pTEV5, where *E. coli* strain DH5α/F' (New England Biolabs) was used.

PCR conditions and sample workup. Specific sequences were amplified using oligonucleotides synthesized by Integrated DNA Technologies (IDT), unless otherwise stated. rTEVLink oligonucleotides used in the construction of the pTEV series of plasmids were synthesized by the UW-Madison Biotechnology Center (UWBC). DNA cleanup reactions were performed with the QIAquick PCR Purification Kit (Qiagen). Gel extractions were done with the QIAquick Gel Extraction Kit.

Plasmid DNA isolation and transformation. The Wizard® *Plus* SV Minipreps DNA Purification System (Promega) was used for isolation of plasmid DNA. Plasmids pTEV3 and pTEV5 (see below) were sequenced using primers 5'–TTAATACGACTCACTATAGG–3' and 5'–CCATTCGCCAATCCGGAT–3'. Plasmids were introduced into the desired strains by transformation, using described protocols (Inoue *et al*., 1990)

DNA sequencing. Non-radioactive BigDye® protocols (ABI PRISM) were used to sequence DNA samples. After the reactions were completed, samples were purified using the CleanSEQ reaction procedure (Agencourt Biotechnology) and resolved at the UWBC.

Protein purification. Cell cultures were grown at 37°C in lysogenic broth (LB) (Bertani, 1951; Bertani, 2004) supplemented with ampicillin (100 μg/ml) to a cell density (OD_{650}) of 0.6 – 0.7, gene expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG, 0.3 mM) followed by an 18-h incubation period at 37°C with shaking (180 rpm). After induction, cells were harvested by 10,000 x *g* for 15 min at 4°C using a Beckman-Coulter Avanti™ J-20 XPI centrifuge and a JLA 8.1 rotor, and disrupted by treating the cell slurry with lysozyme (1 mg/ml) and BugBuster® (Novagen) at 4 °C.

Unbroken cells and membranous material were removed by centrifugation at 39,000 x *g* for 20 min. His-tagged proteins were purified by metal affinity chromatography using Novagen's His-Bind® Kit following manufacturer's instructions. His_6-MBP- PrpD protein was purified using Amylose Resin High Flow (Bio-Rad Laboratories) and manufacturer's instructions and visualized on a 12% SDS-PAGE (Laemmli, 1970) polyacrylamide gel stained with Coomassie Blue (Sasse, 1991). Protein concentrations were determined using a commercially available kit for Bradford assays (Bio-Rad Laboratories) measuring absorbance at 595 nm (Bradford, 1976).

Recombinant protein digestion with the rTEV protease. Recombinant protein was mixed with His_6-rTEV protease at a 50:1 mg:mg ratio. The mixture was dialyzed overnight at 4°C in 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (Tris•HCl, 20 mM, pH 7.9 at 25ºC), NaCl (200 mM), and dithiothreitol (DTT, 5 mm). Buffer exchanges were done with Tris•HCl (20 mM, pH 7.9 at 25ºC), and NaCl (400 mM) to remove DTT from the reaction. Cleaved protein was then purified from uncleaved protein and the cleaved tag with Novagen His-Bind® Resin following manufacturer's instructions.

REFERENCES

Bertani, G. (1951). Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* **62,**293-300.

Bertani, G. (2004). Lysogeny at mid-twentieth century: P1, P2, and other experimental systems.. *J. Bacteriol.* **186,**596-600.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**,248-255.

Inoue, H., Nojima, H. & Okayama, H. (1990). High efficiency transformation of *Escherichia coli* with plasmids. *Gene* **96**,23-28.

Laemmli, U. K. (1970). Cleavage and structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**,680-685.

SasseJ. (1991). Detection of proteins. In *Current Protocols in Molecular Biology*, pp10.16.11-10.16.18. Edited by F. A. Ausubel, R. Bent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley Interscience.